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14. ABSTRACT
Our original goal was to correlate exosomal miRNA profiles within circulating tumor-derived exosomes with cancer diagnosis, characterization of circulating tumor-derived vesicles and development of methodology for their specific isolation were essential for specific analyses of tumor-derived exosomal cargoes. Our discovery of exosomal microRNA in cancer patients focused on miRNAs, previously demonstrated in tumor biopsies or ovarian tumor cells. We have observed that while some miRNAs that are up-regulated with the tumor or also up-regulated in their exosomes, some tumor-up-regulated miRNAs are not up-regulated within exosomes. Further, certain miRNAs down-regulated within the tumor are up-regulated in exosomes. We demonstrate that miRNA signatures derived from ovarian tumor exosomes exhibit some miRNAs that are undetectable within the tumor. These findings demonstrate the highly selective nature of miRNA "packaging." Although the exosomal miRNA signatures for ovarian cancer patients appear to be similar regardless of stage, significant differences among early (Stage I and II) and late stage (Stage III) ovarian cancer were demonstrated: increase expression level and elevated expression of three specific miRNAs within exosomes. Exosomes from ovarian cancer patients were demonstrated to possess elevated levels of RNA, including the presence of specific long-noncoding RNA.

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INTRODUCTION

Ovarian cancer remains the fourth leading cause of cancer-related deaths in women, despite progress in the understanding and treatment of ovarian cancer. Annually, in the United States, there are more than 25,500 new cases and 15,310 deaths. [1,2] Most women with ovarian cancer are diagnosed at an advanced stage, with 75% of cases diagnosed with extra-ovarian disease. This late diagnosis may reflect the inaccessibility of the ovaries and the lack of early symptoms.[3] The anatomical location of the ovaries results in minimal interference with vital structures or local irritation, making the diagnosis of ovarian cancer difficult, until regional and distant metastases have occurred.

Although ovarian cancer accounts for only a third of gynecologic cancers, it results in 55% of the deaths from gynecologic malignancies and 6% of all cancer deaths in women.[4,5] Long-term survival has not changed significantly in the last two decades, largely due to inadequacy of diagnostic approaches, which only detects well-established overt cancers. Stage I ovarian cancer can be cured in 90% of cases, while five-year survival for patients with advanced disease (Stage III and IV) is less than 21%. In comparison with other cancers associated with women, 73% of endometrial cancers, 55% of breast cancers and 50% of cervical cancers are diagnosed as Stage I, while only 23% of ovarian cancers are diagnosed at an early stage.[6] Thus, prospects for significant improvement in ovarian cancer survival reside in early diagnosis of disease.

The only biomarker currently approved for ovarian cancer detection is CA125 and its quantitation by ELISA has been the "gold standard" for detection of ovarian cancer, since its introduction in 1983.[7] Assessment of CA125 is typically used in disease management, both for disease detection as well as monitoring for disease recurrence; however, the use of CA125 is limited with regard to early stage cancer detection (sensitivity from 50–60%).[8] CA125 quantitation is only approved for and consistently proven for remission monitoring. CA125 is neither sensitive nor specific for de novo ovarian cancer detection, since it is elevated in >50% of women with stage I disease, although it is elevated in more than 80% of patients with advanced stage ovarian cancer. CA125 has poor specificity, which is shown by its elevation in association with benign and malignant breast and colon disease, peritoneal irritants, and benign gynecologic diseases, among others. Significant effort has been expended in the recent years for identifying potential markers that might substitute or complement CA125 in disease management or ultimately in the design of screening strategies.[9]

To address these problems, new technologies are being investigated. New strategies that facilitate proteomic analysis by dramatically simplifying the pre-analytical sample separation and coupling with mass spectrometry (MS) have been introduced for biomarker discovery research. Surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS) has received much attention for its use in resolving proteins in biological specimens by binding to biochemically distinct ProteinChip arrays. In LabCorp's technology, 4 serum proteins are examined by ELISA, while Correlogic Systems and Ciphergen Biosystems use mass spectrometry of 7 specific serum components or general peptide patterns in patient serum to define the presence of cancer. SELDI-TOF-MS profiling has been successfully used to differentiate ovarian, breast, prostate, and liver cancer from controls.[10] SELDI-TOF-MS profiling of serum was significantly better than the current standard serum biomarker CA125 at distinguishing patients with ovarian cancer from those with benign ovarian disease and from healthy controls.[11] Studies have shown that the selection of a combination of multiple proteins resolved by SELDI-TOF-MS may become a potential diagnostic approach. An effective screening test for ovarian cancer needs to achieve a high sensitivity and specificity and currently, different proteomic technologies as well as the computational analytic tools used to discern peaks generate different findings. These initial studies on SELDI-TOF-MS profiling insights are promising, and the concept is reproducible in a series of different backgrounds; however, translating this approach into a routine diagnostic test remains difficult.

Jacobs and Menon calculated that to be an effective screening test, an assay needs to achieve a minimum of 99.6% specificity.[12] To achieve this level of specificity, multiple components of the tumor's characteristics will need to be incorporated into new diagnostic tests for effective detection because of the multifactorial nature of ovarian cancer. A drawback of mass spectrometry techniques is that some

samples of importance may be masked by more abundant proteins in the MS as well as in the analysis of the spectrometric output. Prepurification by a number of techniques such as high-performance liquid chromatography and positive or negative selection through affinity binding can remove particular groups of proteins. The greatest challenge in most current mass spectrometry approaches is the dynamic range rather than sensitivity. While removal of prevalent proteins or peptides can greatly increase the informational content that can be acquired from particular samples, prevalent proteins such as albumin can function as carriers of protein subsets of diagnostic significance. Additional studies with larger samples sizes and careful blinding of the independent validation sets are needed before any consideration of application of this platform for screening for ovarian cancer or any other indication should be considered.

One general characteristic of tumors is their ability to release or shed intact, vesicular portions of the plasma membrane (termed membrane fragments, membrane vesicles, microvesicles or exosomes), which was initially described by us.[13] Exosomes are described as microvesicles containing 5′-nucleotidase activity that are released from neoplastic cell lines. These small vesicles (50-100nm in diameter), which were present inside large multivesicular endosomes, contained transferrin receptors, a marker that is used to follow endocytosis and the recycling of cell-surface proteins, that had been internalized from the plasma membrane.[14,15] The precise mechanisms of exosome release/shedding remain unclear; however, this release is an energy-requiring phenomenon, modulated by extracellular signals. They appear to form by invagination and budding from the limiting membrane of late endosomes, resulting in vesicles that contain cytosol and that expose the extracellular domain of transferrin receptors at their surface. Using electron microscopy, studies have shown fusion profiles of multivesicular endosomes with the plasma membrane, leading to the secretion of the internal vesicles into the extracellular environment. The rate of exosome release is significantly increased in most neoplastic cells and occurs continuously.[16] Increased release of exosomes and their accumulation appear to be important in the malignant transformation process. In addition to cancer cells, the release of exosomes was also demonstrated to be associated with cells of embryonic origin (such as the placenta) and activated lymphoid cells.[17-20] Although extracellular shedding of exosomes occurs in other types of cells, under specific physiological conditions, the accumulation of exosomes from non-neoplastic cells is rarely observed, in vivo.[17,21] In contrast, exosomes released by tumor cells accumulate in biologic fluids, including in sera, ascites, and pleural fluids. Exosome release and its accumulation appear to be important features of the malignant transformation. Shed tumor derived exosomes do not mirror the general composition of the plasma membrane of the originating tumor cell, but represent ‘micromaps,’ with enhanced expression of tumor antigens.[17,22]

The release of exosomes appears to be important features of intercellular communication. Since released exosomes express molecules with biologic activity (such as Fas ligand, PD-1, MICA/B, mdr1, MMPs, CD44, and autoreactive antigens),[23-26] the ability of these microvesicles to modulate lymphocyte and monocyte functions have been analyzed in several models. It has been theorized that these released exosomes modulate lymphocyte functions by mimicking “activation induced cell death” (AICD).[27,28] Lymphoid cells appear to release exosomes following activation and these appear to play an essential role in immunoregulation, by preventing excessive immune responses and the development of autoimmunity.[30] It was postulated that exosome release by tumor cells is a re-expression of the fetal cell exosomes and that both constituted pathways to circumvent immunosurveillance.[30]

miRNAs are small endogenous noncoding RNA gene products about 22 nucleotides (nt) long that regulate gene expression in a sequence-specific manner and are found in diverse organisms. With >300 already identified, the human genome may contain up to 1,000 miRNAs. miRNA play key roles in regulating the translation and degradation of messenger RNAs through base pairing to partially complementary sites, predominately in the untranslated region of the message.[31-33] miRNAs are expressed as long precursor RNAs. Drosha, an RNase III endonuclease, is responsible for processing primary miRNAs in the nucleus and releasing ~70nt precursor miRNAs.[34,35] Drosha associates with the dsRNA-binding protein DGCR8 in human to form the microprocessor complex. Precursor miRNAs are

transported to the cytoplasm by exportin-5 and cleaved by the RNase III endonuclease Dicer, releasing 17–24nt mature dsmiRNA.[36,37] One strand of the miRNA duplex is subsequently incorporated into the effector complex RNA-induced silencing complex (RISC) that mediates target gene expression. Argonaute2, a key component of RISC, may function as an endonuclease that cleaves target mRNAs.

While the biological functions of most miRNAs are not yet fully understood, it has been suggested that the miRNAs are involved in various biological processes, including cell proliferation, cell death, stress resistance, and fat metabolism, through the regulation of gene expression.[38] As potential clinical diagnostic tools miRNAs have been shown to be important and accurate determinants for many if not all cancers.[39] Increasing evidence shows that expression of miRNA genes is deregulated in human cancer. The expression of miRNAs is highly specific for tissues and developmental stages and has allowed recently for molecular classification of tumors. To date, all tumors analysed by miRNA profiling have shown significantly different miRNA profiles compared with normal cells from the same tissue. Flow-cytometric miRNA profiling demonstrated that miRNA-expression profiles classify human cancers according to the developmental lineage and differentiation state of the tumors. Specific over- or underexpression has been shown to correlate with particular tumor types. miRNA overexpression could result in down-regulation of tumor suppressor genes, whereas their underexpression could lead to oncogene up-regulation. Using large-scale microarray analysis, cancer cells showed distinct miRNA profiles compared with normal cells with 36 of the 228 miRNA genes overexpressed and 21 downregulated in cancer cells versus normal cells.[40] Hierarchical clustering analyses showed that this miRNA signature enabled the tumour samples to be grouped on the basis of their tissue of origin. Genome-wide profiling studies have been performed on various cancer types, including CLL,[41] breast cancer,[42] glioblastoma,[43] thyroid papillary carcinoma,[44] hepatocellular carcinoma,[45] ovarian cancer,[46] colon cancer,[47] and endocrine pancreatic tumours.[48] In a study of 104 matched pairs of primary cancerous and non-cancerous ovarian tissue, 43 differentially expressed miRNAs were observed; 28 were downregulated and 15 were overexpressed in tumors.[49]

Statistical analyses of microarray data obtained by two different methods, significance analysis of microarrays (SAM) and prediction analysis of microarrays (PAM) from six solid tumours (ovarian, breast, colon, gastric and prostate carcinomas and endocrine pancreatic tumours), demonstrated a common signature composed of 21 miRNAs differentially expressed in at least three tumor types.[50] At the top of the list were miR-21, which was overexpressed in six types of cancer cells, and miR-17-5p and miR-191, which were overexpressed in five. As the embryological origin of the analysed tumors was different, the significance of such findings could be that these common miRNAs participate in fundamental signalling pathways altered in many types of tumor. Supporting the function of these genes in tumorigenesis, it was found that the predicted targets for the differentially expressed miRNAs are significantly enriched for those that target known tumor suppressors and oncogenes.[51] Furthermore, miR-21, the only miRNA overexpressed in all six types of cancer analyzed was shown to directly target the tumor suppressor PTEN, which encodes a phosphatase inhibiting growth and/or survival pathways. The function of PTEN is altered in advanced tumors of various types, including breast, ovarian, gastric and prostate.[52]

KEY RESEARCH ACCOMPLISHMENTS

Over the past 12-month period, we have generated multiple accomplishments leading to significant improvements of ovarian cancer diagnostic and prognostic markers.

1. Expanded quantitation and characterization of circulating vesicles in ovarian cancer patients
2. Development of asymmetric field flow fractionation from isolation of ovarian tumor exosomes.
3. Next generation sequencing analysis of tumor-derived exosomal RNA
4. Specific microRNAs linked with various stages of ovarian cancer
5. Exosomal miRNAs linked with other solid tumors
6. Exosomes and their cargoes associated with non-solid tumors
7. Defined the actions of tumor-derived exosomes on target cells

As the original aim was to define the utility of exosomal miRNA profiles as diagnostic biomarkers by correlating specific miRNAs associated with circulating tumor-derived exosomes with diagnosis (stage and grade), achievement of the overall goals of this Sponsored Research Agreement were essential to enable the specific analyses of tumor-derived exosomes and their contents from those exosomes derived from normal cells within the peripheral circulation (ie, reduction of "noise" to enhance the signal to noise ratio). We have continued to increase the number of ovarian cancer patients at each stage evaluated to define the microRNA signatures of the tumors. Our original discovery of exosomal microRNA in cancer patients focused on a small number of miRNAs that previous groups demonstrated to be diagnostic using tumor biopsies or cultured ovarian tumor cells; however, we have observed that while some miRNAs that are up-regulated with the tumor or also up-regulated in their exosomes, some tumor-up-regulated miRNAs are not up-regulated within exosomes. In addition, we observed that certain miRNAs that exhibit down-regulation within the tumor are up-regulated in exosomes. We have further investigated this finding and demonstrate that in many cases, miRNA signatures derived from ovarian tumor cell exosomes exhibit some miRNAs that are undetectable within the tumor. Our findings in multiple ovarian tumor cell lines demonstrate commonality in these miRNAs. This finding is in addition to cellular down-regulated miRNAs being up- regulated in exosomes derived from the same cells. These findings demonstrate the highly selective nature of miRNA "packaging" into exosomes. Based on these findings, previous data are being re-evaluated to incorporate those miRNAs (not appearing in the tumor cells) into the "diagnostic" signature. Although the exosomal miRNA signatures for ovarian cancer patients appear to be similar regardless of stage, work within the past 12 months has demonstrated significant differences among early (Stage I and II) and late stage (Stage III) ovarian cancer: general increase expression level and the elevated expression of three specific miRNAs within exosomes. We expanded the identification of exosomal miRNA using the Life Technologies Ion Torrent with both TLDA Profile A and B cards.

While not included with the original objectives of this study, our work demonstrated that exosomes from ovarian cancer patients possessed elevated levels of RNA (less 400 nt). Our proposal focused on the miRNA populations; however, during the past 12 months, we further analyzed "larger" fragments of RNA. We demonstrated the presence of multiple mRNA fragments using next generating sequencing.

Summaries of the findings for these key research accomplishments are presented on the following pages.

REPORTABLE OUTCOMES

Expanded quantitation and characterization of circulating vesicles in ovarian cancer patients.
A major limitation of DLS and SPA, as well as standard NTA, is that while they can objectively define the vesicle size range, they cannot define the "phenotype" of these vesicles. Using the NanoSight equipped with the 405-nm blue-violet laser and more sensitive camera to detect fluorescent particles, quantum dots attached to antibodies can be used identify specific subsets of vesicles. The instrument was

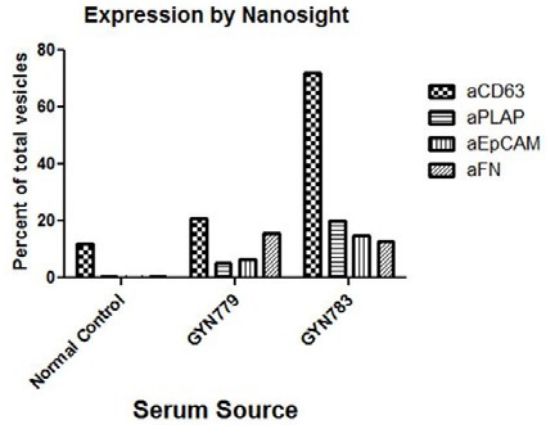


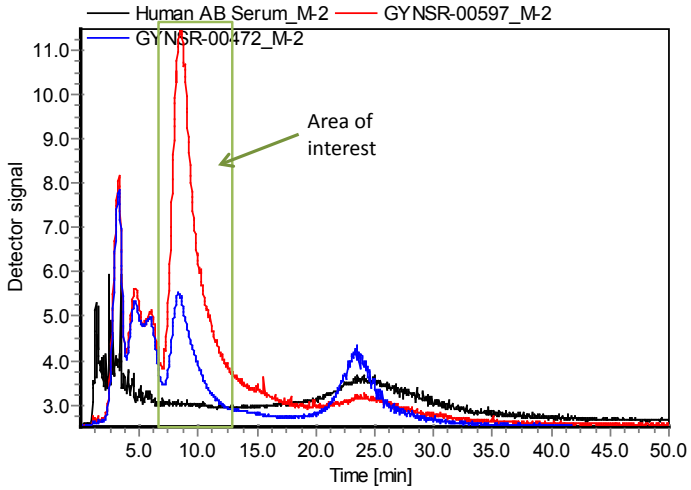
Figure: Expression of specific markers on circulating exosomes defined by Nanosight in fluorescence-mode (compared to total exosomes defined in light scatter mode)

initially calibrated using 100nm and 200nm fluorescent beads, which can be easily discriminated. Antibodies reactive with either CD63 (exosomes marker), EpCAM PLAP, or fetal fibronectin (all previously demonstrated to be markers of vesicles derived from ovarian tumors) were conjugated with quantum dots.

The labeled vesicle samples were analyzed on the NanoSight, first in light scatter mode and then in fluorescence mode. Thus, it is now possible to visualize and quantitate those circulating exosomes derived specifically from the tumor.

Development of asymmetric field flow fractionation from isolation of ovarian tumor exosomes

Separation by AFFF and analysis of vesicular fraction from cancer patients (GYNSR597 and GYNSR472) compared to normal controls by 90° angle static light scattering



Asymmetric Field Flow Fractionation (AFFF) is new technology designed for the separation of synthetic nanoparticles. AFFF is a one-phase chromatography technique that generates high-resolution separation within a flow against which a perpendicular force field is applied. The flow and sample are positioned within a channel consisting of two plates that are separated by a spacer foil, with a thickness of 100 to 500 µm. Within the channel, the upper plate is impermeable, while the lower channel plate is constructed of a porous frit material. In the flow channel, a parabolic flow is generated by the laminar flow of the buffer: the stream moves slower closer to the boundary edges than it does at the center of the channel flow. When the perpendicular force field is applied to the flowing, laminar stream, the analytes are driven towards the boundary layer the so-called "accumulation wall" of the channel. Diffusion associated with Brownian motion generates a counteracting motion. The velocity gradient flowing inside the channel separates different sizes of particles. Smaller particles migrate more rapidly through the channel than the larger particles. Since there is no gel media, no shearing forces are applied to the sampel. The entire separation is gentle, rapid, and non-destructive without a stationary phase that may interact, degrade, or alter the sample. We have completed the proof of concept for this approach. We demonstrate the unique presence of the 9-10 minute vesicle peak in ovarian cancer patients. This material is undetectable in all normal.

We have developed a non-invasive, high throughput, blood-based exosome platform to address a critical issue - the need to evaluate in real time the state of tumors in individual patients prior to (diagnostic) and in response to therapy. This development addresses the need for biomarkers that serve as predictors or surrogates of therapeutic efficacy. Our hypothesis is that circulating tumor-derived microvesicles and their components can report on the presence, extent and therapeutic responses of ovarian tumors. This proposed commercialization study will provide a comprehensive profile of vesicle populations in blood from ovarian cancer patients by the novel technique of asymmetric field flow fractionation. These analyses will be applied to detect levels and critical characteristics of tumor-derived exosomes in the peripheral circulation to define transcriptome/protein content, which can provide information as to driver mechanisms in individual ovarian tumors. This will correlate specific exosome biomarkers with tumor size and genotype, as well as therapeutic responses and recurrence, survival and clinical status.

This study represents a new paradigm for monitoring ovarian cancer patients. For these patients, exosomal analyses are predicted to facilitate the decision-tree of clinical care. Our goal is to monitor blood-derived exosomal proteomic profiles and transcriptome profiles to identify pathway-response changes in tumors that signal recurrence of ovarian cancer and resistance to treatment. This information will have wide applicability in determining the genotype/phenotype of many types of cancers using easily accessible blood samples and can be combined with other types of biomarker information to give a rapid read-out on response to therapy and mechanisms of resistance to therapy. While soluble individual proteins and nucleic acids are rapidly degraded in the blood, these components when associated with exosomes are stable over time and mirror the original tumors. A current major challenge and opportunity is the development of methods for rapidly

determining the abundance and composition of circulating tumor-specific exosomes from clinical samples. We have developed the high-throughput methodology for isolation of specific tumor-derived exosomes. This approach allows the profiling of tumor-derived exosomes, with unique protein expression levels identifying ovarian cancerfrom host cell exosomes and the utility of exosome profiling to follow cancer treatment efficacy. This approach will quantitate both exosome number and composition as indicators of therapeutic efficacy in clinical trials.

Specific microRNAs linked with various stages of ovarian cancer

Initial analyses were performed by cancer-specific arrays from SABiosciences. The small RNA-enriched fraction was extracted from the isolated exosomes. Using specific primers, presence and expression level of mature miRNAs was analyzed by TaqMan miRNA Assay under conditions defined by the supplier. Single-stranded cDNA will be synthesized from 5.5ng of total RNA in 15µl reaction volume by using the TaqMan MicroRNA Reverse Transcription Kit (AB). The reactions will be incubated first at 16°C for 30 min and then at 42°C for 30 min. The reactions will be inactivated by incubation at 85°C for 5 min. Each cDNA generated will be amplified by quantitative PCR by using sequence-specific primers from the TaqMan microRNA Assays Human Panel on a Agilent M3005P. The 20µl PCR mix will include 10µl of 2× Universal PCR Master Mix, 2µl of each 10× TaqMan MicroRNA Assay Mix and 1.5µl of reverse transcription (RT) product. The reactions will be incubated at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The threshold cycle (C_T) is defined as the fractional cycle number at which the fluorescence passes the fixed threshold (0.2). All signals with $C_T \geq 37.9$ will be manually set to undetermined. The relative quantity (RQ) of the target miRNAs will be estimated by the ΔC_T study by using as reference (exogenous control) for each preparation. Each sample will be run in duplicate and each PCR experiment will include two non-template control wells. From these analyses, exosomal miRNAs that were previously reported to be specifically up-regulated in ovarian cancer cells were examined. Similarly, those exosomal miRNAs shown to be specifically down-regulated were examined.

One objective of this study was to define miRNA signatures that might differentiate early and late stage ovarian cancer. For these studies, serum specimens of patients with Stage I, II or III serous papillary adenocarcinoma of the ovary were evaluated. The small RNA-enriched fraction was extracted from the isolated exosomes. Using specific primers, presence and expression level of mature miRNAs was analyzed by TaqMan miRNA Assay (Applied Biosystems) under conditions defined by the supplier. LMW RNA was isolated from exosomes isolated from 1ml of sera using the mirVana miRNA Extraction Kit and quantified by the RiboGreen kit. Single-stranded cDNA will be synthesized from 5.5ng of total RNA in 15µl reaction volume by using the TaqMan MicroRNA Reverse Transcription Kit (AB). The reactions will be incubated first at 16°C for 30 min and then at 42°C for 30 min. The reactions will be inactivated by incubation at 85°C for 5 min. Each cDNA generated will be amplified by quantitative PCR by using sequence-specific primers from the TaqMan microRNA Assays Human Panel on a Agilent M3005P. The 20µl PCR mix will include 10µl of 2× Universal PCR Master Mix, 2µl of each 10× TaqMan MicroRNA Assay Mix and 1.5µl of reverse transcription (RT) product. The reactions will be incubated at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The threshold cycle (C_T) is defined as the fractional cycle number at which the fluorescence passes the fixed threshold (0.2). All signals with $C_T \geq 37.9$ will be manually set to undetermined. Using the Cancer miRNA qRT-PCR array, while the heat maps were similar across stages, the advanced ovarian cancer patients generally expressed enhanced miRNA expression. This elevated expression within exosomes from Stage III ovarian cancer patients was also observed with the scatter plot.

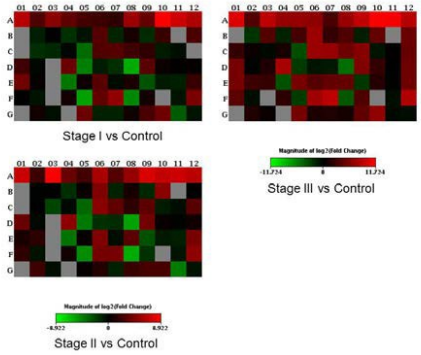


Figure : Heat maps of microRNA arrays examining the expression of miRNA in exosomes isolated from ovarian cancer patients at various stages versus controls.

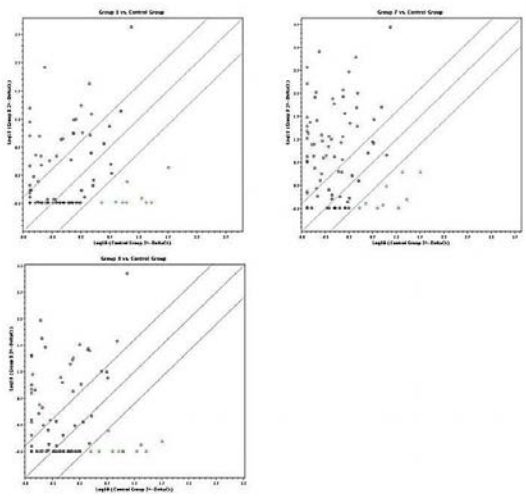


Figure : Scatter plot of miRNA expression associated with exosomes from Stage I (Group 1), Stage II (Group 2) or Stage III (Group 3) ovarian cancer

Comparisons between these populations of exosomes derived from cancer patients were not significantly different for most of these miRNAs. The similarity across the stages of ovarian cancer may result from the standardization of starting exosomal small RNA quantities and the normalization of the resulting array data. Despite this standardization and normalization, the profiles obtained with exosomal miRNA from patients with advanced ovarian cancer (Stage III) exhibited some distinct differences. While exosomes derived from all patients with ovarian cancer exhibited similarities, these were distinguished from patients without cancer (both controls and benign disease) and patients with advanced ovarian cancer exhibit a unique signature.

In addition to exosomal miRNA analyses in ovarian tumors, the analyses were expanded to breast cancer (Attachment 1), bladder, colon and pancreatic cancers (Attachment 2). Tumor specific profiles were observed with each tumor type.

Next generation sequencing analysis of tumor-derived exosomal RNA

The current hypothesis for the stability of circulating RNA is that they are released from cells in membranous vesicles. Recent data confirm that extracellular RNA can exist in four forms: free RNA, Argonaut 2-bound RNA, high-density lipoprotein-bound RNA and vesicle-associated RNA. This review focuses on RNA associated with extracellular vesicles. These extracellular vesicles are generated constitutively by most, but not all, cell types and contain both mRNAs and non-coding RNA. The ability of extracellular vesicles to transfer genetic information may facilitate cancer spread by delivering genetic material and oncogenic proteins. RNA profiles of extracellular vesicles differ from that of cellular RNA, since vesicles contain primarily small RNA, such as mRNA and microRNA, in the absence of ribosomal RNA. The presences of circulating RNAs have been extensively investigated, despite the presence of highly stable RNases, which should degrade any free RNA. The majority of the circulating RNAs have been defined as microRNAs based on the molecular weight. Studies also demonstrated that microRNAs not only have high stability in body fluids, but also survive in the unfavorable physiological conditions such as freeze-thawing, extreme variations in pH and long time at room temperature. Whereas adding detergents, such as Triton X or SDS, to serum or plasma makes microRNAs easily degradation by RNases. The results indicate there are at least two approaches responsible for the stability of extracellular microRNAs: be packaged in membrane-encapsulated vesicle and be protected by RNA-binding proteins.

The stability of extracellular microRNAs has been hypothesized to be due to the formation of the RNA-vesicle. During RISC disassembly in the cytoplasm, some microRNAs are found to be sorted into MVBs, which are commonly considered to form exosomes by fusion with the plasma membrane. Both exosome and microvesicle can easily translocate across the cell membrane, which makes microRNAs enter

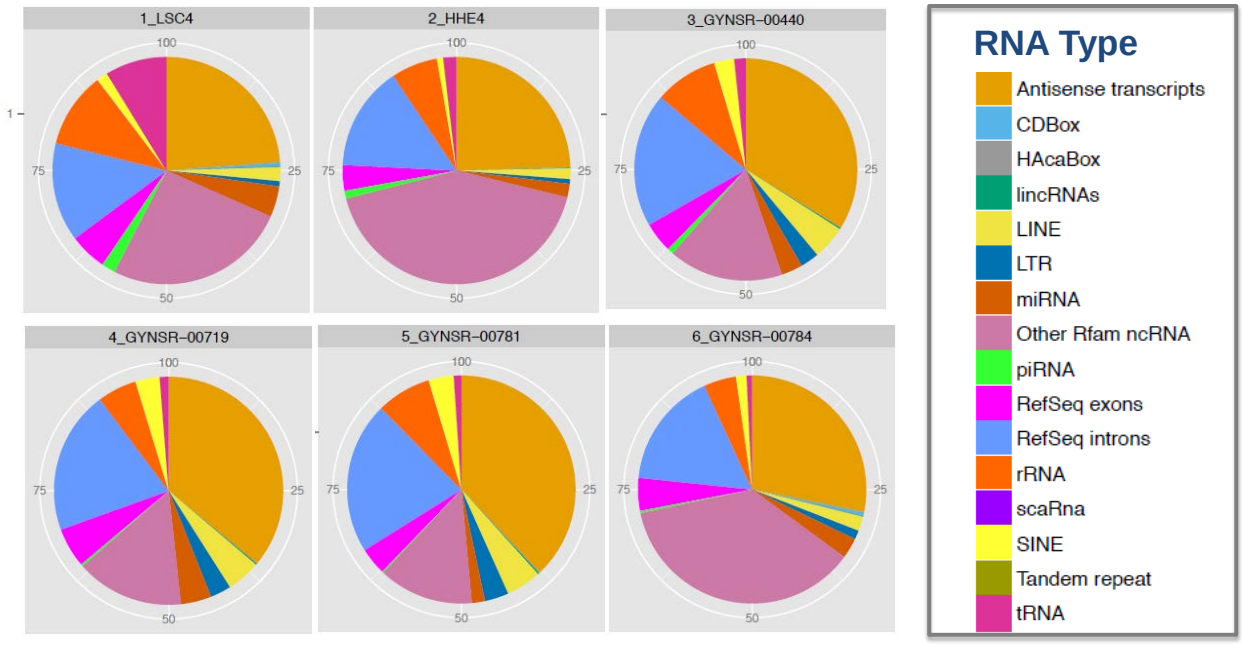
recipient cells easily and mediate cell-to-cell communication. Our studies have indicated that many of RNAs enriched in the extracellular vesicles may not be abundant, or even detectable, in the originating cell or highly expressed within the cell and low or absent within extracellular vesicles, indicating sorting of specific RNAs into extracellular vesicles. These released microRNAs can be classified in three categories based on the ratio between the amount of microRNA released from the cells and the amount retained in the cell. The first group is selectively released microRNAs, which are characterized by being primarily released from tumor cells with relatively low concentrations remaining in the cell. In contrast, normal cells do not release appreciable quantities of these microRNAs. An additional group of released microRNAs are those released in equal levels as they appear within the cell, termed neutrally released microRNA. These neutrally released microRNAs include miR16 and miR21, where the abundance in extracellular vesicles reflects increased abundance in the tumor cells. The selectivity of release of specific microRNAs differs depending on the cell type. Selectivity appears to be influenced by malignant transformation. Breast and ovarian tumor cells have been demonstrated to release >99% of miR451 and miR1246 produced by the cells. These selectively released microRNAs have been linked to the malignant phenotype. MiR451has been identified as a tumor suppressor, defining proliferation and cell polarity. miR451 has also been shown to induce chemosensitivity. miR1246 induces p53-dependent apoptosis triggered by DNA damage. The changes in the release of cancer-related microRNAs may suggest a role for selective microRNA export in malignant transformation, and it may provide a cancer signature within the exported, circulating microRNA population.

While the mechanism of this selective sorting is unclear, some have postulated this selectivity relates to microRNA/RNA-induced silencing complex (RISC) components. Extracellular vesicles contain components of the microRNA/RISC, such as Argonaut 2, together with several RNA-binding proteins known to regulate RNA traffic between the nucleus and the cytoplasm. It can be therefore hypothesized that, during vesicle biogenesis, these RNA binding proteins regulate the accumulation of selected RNAs within extracellular vesicles. Studies on the transfer of reporter mRNAs and their translation into proteins, demonstrated both in vitro and in vivo, suggest that the mRNA delivered by extracellular vesicles is functional. Extracellular vesicles derived from other tumors such as colorectal, lung, and prostate cancer cells alter the phenotype of normal cells by transferring specific RNA subsets. In contrast, extracellular vesicles released from the surrounding cells may modify cancer cell gene expression. Extracellular vesicles derived from cancer stem cells were shown to contain pro-angiogenic RNAs able to induce a pre-metastatic niche in the lungs, whereas those derived from differentiated cancer cells were not able to induce this niche and their mRNA and microRNA content differs. Extracellular vesicles from cancer stem cells contained miR29a, miR650, and miR151, all associated with tumor invasion and metastases, along with miR19b, miR29c, and miR151, known to be up-regulated in patients with renal carcinomas.

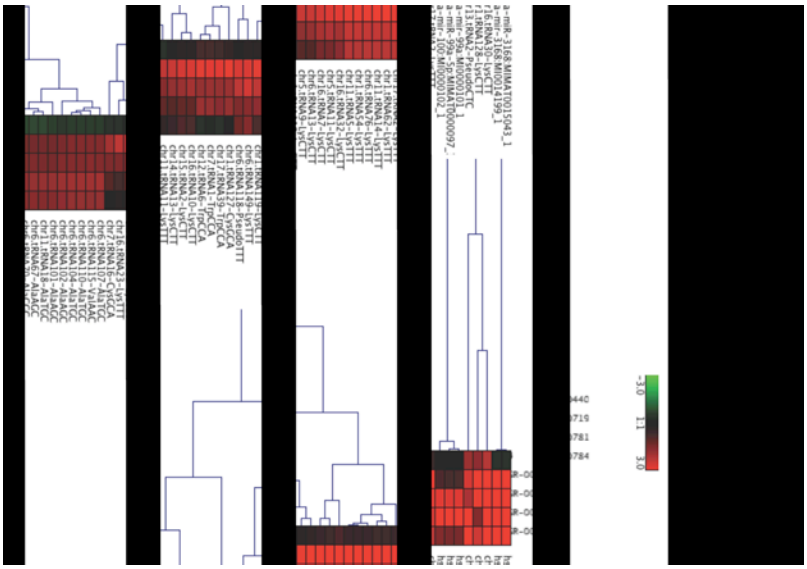
Extracellular vesicles have been isolated and analyzed from both normal healthy individuals and patients with various physiological conditions. We have previously shown that cancer patients and pregnant women exhibit more extracellular vesicles in their blood compared to their normal, healthy counterparts. In pregnant women, the extracellular vesicles are thought to play a role in the maternal–fetal tolerance occurring during pregnancy, as it has been shown that placenta extracellular vesicles suppress T lymphocytes. Most investigations on small RNAs in exosomes have been limited to microRNA; however, next generation sequencing small RNAs in extracellular vesicles is expanding the populations identified. While intracellular microRNAs have been defined in many biological processes, identification of extracellular vesicle-associated microRNAs represents a non-invasive approach to investigate disease-specific microRNA and may provide a method for disease diagnosis. To detect, analyze, and quantitate the RNA signatures of exosomes derived from biologic fluids, several approaches have been used, including microarrays, quantitative real-time PCR, and next-generation sequencing. The development of high detection sensitivity in next generation sequencing technologies has expanded the identification of the exosomal transcriptome, beyond miRNA. While most studies have focused on exosomal microRNAs, we now recognize the presence of numerous other small RNAs within these circulating exosomes, as well as fragments of larger RNAs. These exosomal small non-coding RNAs are <200 nucleotides in length (generally are 20-30 nt). There are three primary populations of small non-coding RNAs, including siRNAs, miRNAs, and piRNAs. Small non-coding RNAs have been shown to be key regulators in development, apoptosis, stem cell self-renewal, differentiation, and cell integrity maintenance. Piwi-interacting RNAs

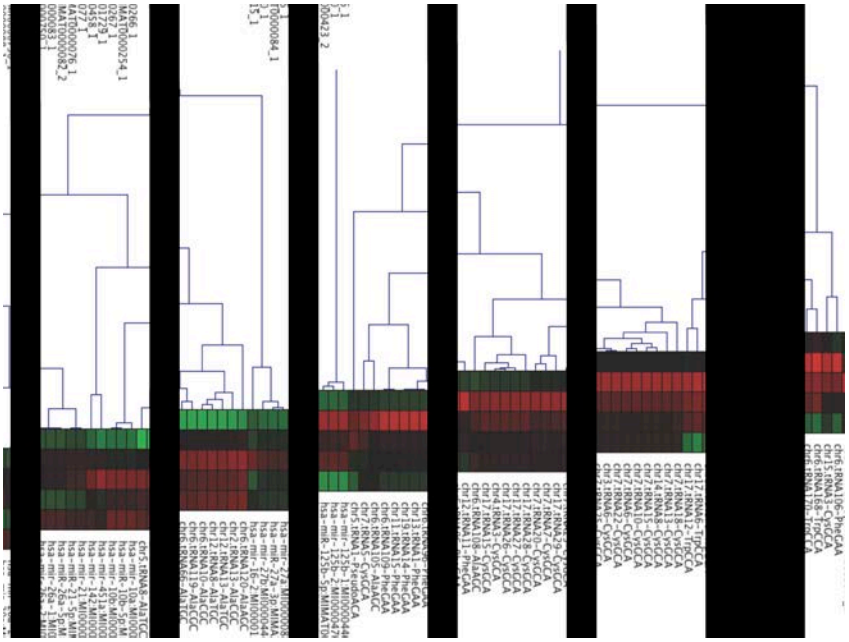
(piRNAs) are generated from intergenic elements, including transposable elements, through Dicer-independent pathways. These piRNAs function through the Piwi-Argonaute sub-family (AGO3, Aubergine, and Piwi), leading to silencing of transposable elements. A link between piRNAs and cancer has been demonstrated in gastric cancers where two aberrantly expressed piRNAs, piRNA-651 and piRNA-823, were found in gastric tumor tissue versus paired normal tissue.

We isolated exosomes from 1ml serum samples of patients with benign ovarian disease and ovarian cancer, as well as a healthy female volunteer. Total RNA was isolated using a phenol-free exosome lysis buffer (SBI). The remaining material was assessed from quality controls by gel electrophoresis and Bioanalyzer. For the resulting exosomal RNA, adapters were initially ligated to the 3' and 5' ends for NGS by standard techniques. This RNA was then subjected to first strand synthesis reverse transcription and amplification with index and PCR. The resulting material was then gel purified. The gel purified material was subjected to NGS. Overall, the exosomal RNA was distributed among antisense transcripts, lincRNAs, LINE, LTR, miRNA, rRNAs, scaRNAs, and tRNAs



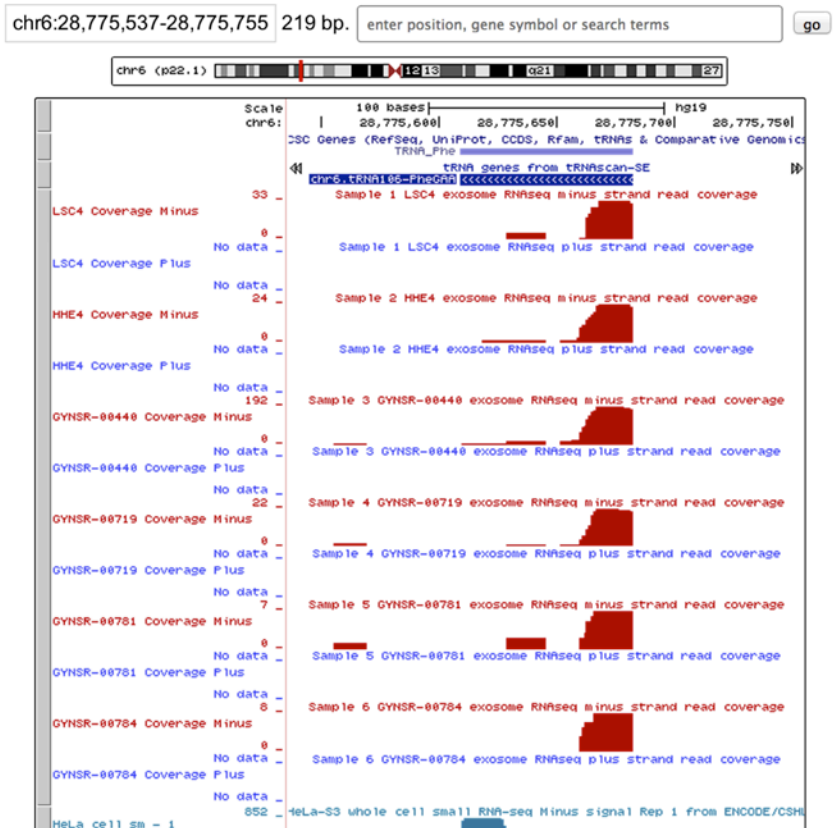
Our analyses of the initial group of ovarian cancer patient samples identified several unique miRNA and tRNA fragments.





Our analyses of the initial group of ovarian cancer patient samples demonstrated a significant enrichment of the 5' end of tRNA-Phe-GAA. While this 5' end fragment is enriched in exosome samples, the 3' prime fragment is in excess abundance in cellular samples.

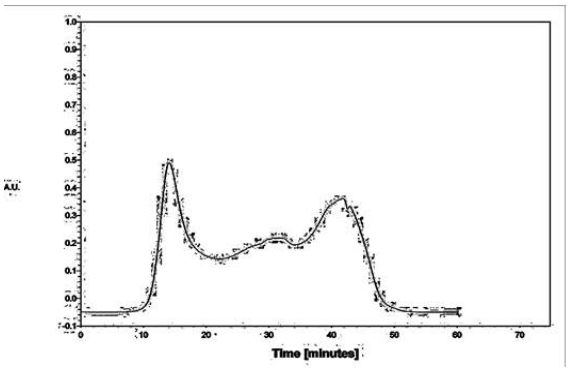
miRNAs of interest. Note 5' end is enriched in exosome samples, whereas 3' end fragment is in excess abundance in ENCODE cellular samples.

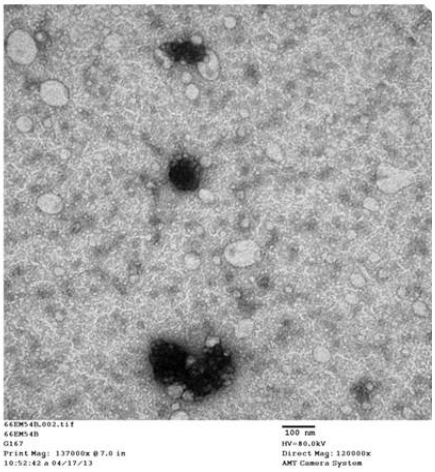


ANALYSES OF EXOSOMES IN NON-SOLID TUMORS

In solid tumors, it has been demonstrated that exosomal signaling plays an essential role in disease progression and therapy resistance. Tumor cells, including AML, release extracellular vesicles that can mediate communication with host components. Our hypothesis is that, in addition to promoting interactions with host immune and stromal components, extracellular vesicles from AML can serve to identify the presence and characteristics of the leukemia. Extracellular vesicles were isolated from 1ml serum samples of patients with AML (n=8) versus normal controls by Sepharose 2B chromatography. The resulting vesicular isolates were characterized by Nanosight for number and size range under light scatter mode and the presence of CD63 under fluorescent mode. The vesicular nature was confirmed by electron microscopy. The presence of exosome and immunological markers was identified by western immunoblotting.

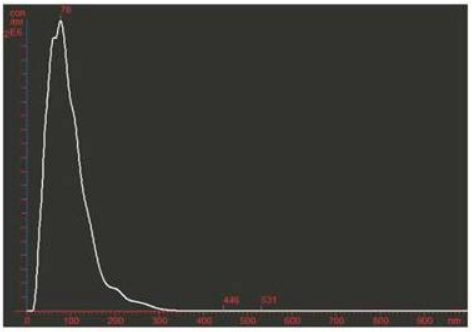
Patients with AML expressed an increased number of circulating extracellular vesicles within their peripheral circulation compared to normal controls ($4.01 \pm 2.11 \times 10^{10}$ for AML versus $1.12 \pm 0.19 \times 10^{10}$, $p = 0.026$). These vesicles exhibited a more uniform diameter than those observed in the control population (mean of 111.6±23.3 nm; mode of 85.3±22.8 nm).



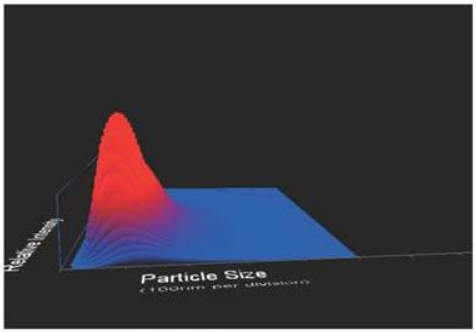


NANOSIGHT

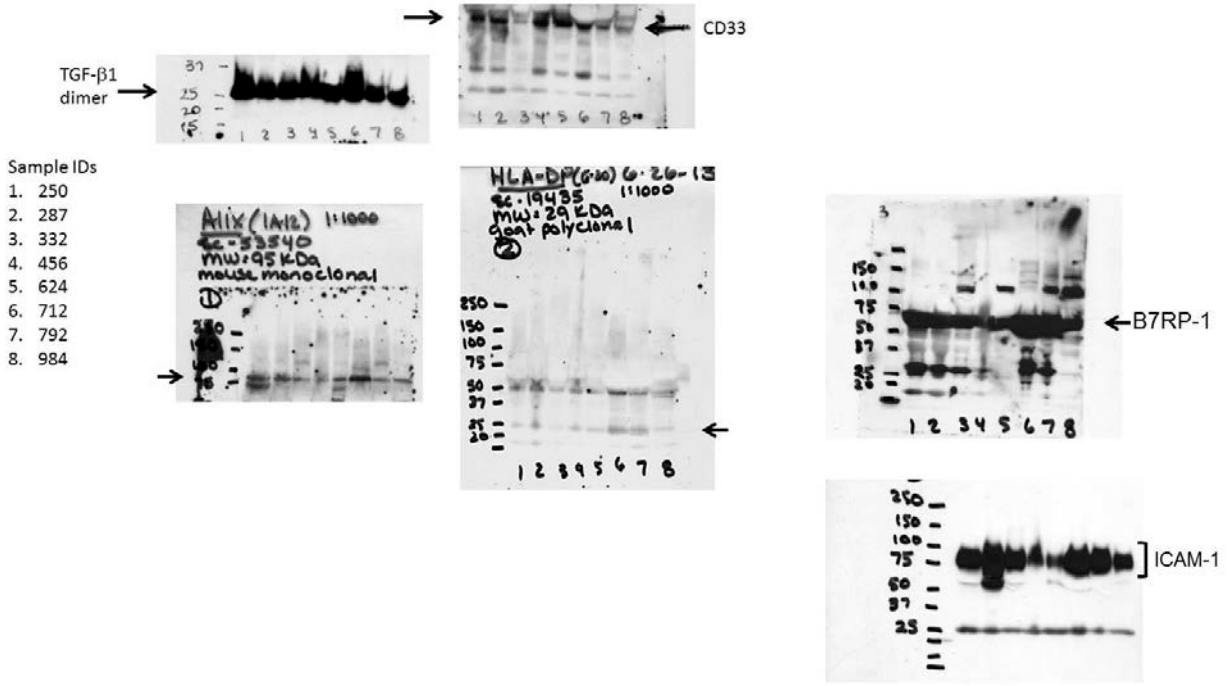
Nanoparticle Tracking Analysis (NTA) Version 2.3 Build 0017



Particle Size / Concentration



Particle Size / Relative Intensity 3D plot



CD63, a marker of exosomes was present over the entire size range. Western immunoblot confirmed the exosome nature of these vesicles based on the expression of CD63 and Alix. In contrast to vesicles derived from normal controls, exosomes from AML patients expressed high levels of the TGF-β, CD33, ICAM-1, B7RP-1 and class-II MHC.

Patients will AML exhibit elevated levels of circulating vesicles that express markers of exosomes. The circulating exosomes also exhibit markers of immunologic status of these patients. Current work is directed at defining whether these vesicles are derived directly from the tumor cells or elicited from normal host components in response to the tumor. In either case, the presence of these exosomes and their associated markers can serve as indicators of tumor presence and disease status.

DEMONSTRATION OF CONSEQUENCES OF TUMOR-DERIVED EXOSOMES OF HOST TARGET CELLS

Many of the miRNAs associated with tumor exosomes have been demonstrated to be associated with regulation of the immune system. For future development of targeted biologic therapeutics, it is essential to define the direct consequences of tumor-derived exosomal miRNA on lymphocyte activation and functions. In an effective immune response, tumor-linked proteins are detected and an anti-tumor response is promoted to eliminate the transformed precursors before they establish malignancy. Effective elimination is characterized by the simultaneous collaboration of innate and adaptive cell-mediated and humoral responses. In the adaptive anti-tumor response, T cells (with cognate TCR) recognize tumor-associated antigens processed/presented on the MHC of antigen-presenting cells (APC), along with subsequent costimulation and cytokine expression for facilitation and maintenance of the response. Elimination of the tumor is accomplished through the activation of cytotoxic T (CTL) cells to induce tumor cell apoptosis, activation of CD4+T cells to promote both cellular and humoral responses through stimulation of APC presentation of antigens to CTL and activation of B cells to produce antigen-specific antibodies that enhance tumor cell

uptake by APCs. The primary anti-tumor response is facilitated by the cellular arm of the adaptive immune system; however, humoral responses to tumour antigens are clearly demonstrated through the production of anti-tumor antibodies. This production of antibodies is presented as elevated IgG in the blood. In ovarian cancer sera, levels of tumor reactive-IgG are elevated, suggesting intact humoral immunity in ovarian cancer patients and an effective humoral anti-tumor response. However, in the midst of this IgG-laden environment, ovarian tumors continue to thrive. A key factor in the progression of transformed cells to malignancy is the tumor microenvironment. The tumor microenvironment consists of a number of cellular participants, including immune cells, which are critical for the suppression of tumor growth. However, the functional activities of these immune cells are often counter-regulated by tumor cell expression and the release of a number of biologic components, which act to promote the growth and metastatic progression of the tumor. One essential biologic component in growth and progression is the tumor derived exosome (TDE). Studies indicate that increased release of exosomes facilitate communication between the tumor's microenvironment and the tumor cell. TDE express tumor-derived antigens; however, they are not molecular duplicates of the plasma membrane of their parental tumor cells; rather, they represent a 'micromap' that displays increased expression of antigens associated with the tumor. TDE are abundantly found in plasma and malignant effusions derived from cancer patients and their presence and expression of tumor-related antigens has been documented to contribute to tumor progression. Progressive effects mediated by TDE have been found to range from regulation of tumor growth to invasion, angiogenesis and metastasis through expression of molecules such as matrix metalloproteinases (MMP-2, MMP-9) and horizontal transfer of growth factor receptors (EGFRvIII). Additionally, TDE have been shown to directly and indirectly modulate the evasion of antitumor responses provided by effector T cells for assisting in progression. Melanoma-derived exosomes have been shown to promote monocyte production of myeloid-derived suppressor cells, which can act to suppress T-cell responses. Ovarian TDE demonstrate induced apoptosis of T cells by enhanced expression of Fas L on the exosomes and CD3zeta suppression on the T cell, while nasopharyngeal TDE have been shown to express increased galectin-9 to induce T cell apoptosis via Tim-3.

Since the activation of B cells and the production of anti-tumor are a hallmark of advanced cancers, this study defined the consequences of tumor-derived exosomes on immature B cells (RA-1) and plasma cells (H929). Tumor exosomes were isolated from patient ascites. The ascites fluids were centrifuged at 400xg for 10 minutes to remove cells and the supernatant was centrifuged at 15,000xg for 20 minutes to remove cell debris. The resulting supernatant was concentrated by ultrafiltration using an Amicon stirred cell with a molecular weight cut-off membrane of 500,000 Daltons (Millipore). This concentrated material was then chromatographically separated using a Sepharose 2B column (2.5x30cm). The void volume fractions were pooled and centrifuged at 100,000 x g for 1 hour at 4°C. The pellet was resuspended in PBS and the protein concentration determined using the DC protein assay. To exclude the possibility of endotoxin contamination in the exosome preparations for ascites-derived vesicles, a LAL assay (Genscript, Piscataway, NJ) was performed to quantify any endotoxin in the vesicle preparations.

To define the effects of tumor-derived exosomes on B cell chemokine and cytokine profiles, their productions by RA-1 and H929 cells were quantified with duplicate arrays, each having duplicate spots for each cytokine using Proteome profiler™ Human Cytokine Antibody Array Panel A Arrays (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. The B cell lines were incubated with 100µg/ml tumor-derived exosomes or untreated for 20 hours. The cytokine array membranes were incubated with 1ml of conditioned media from each sample, diluted 1:3 and 15µl of Cytokine Array Panel A detection antibody at 4°C overnight. The membranes were then washed three times with 20 ml of 1× wash buffer and incubated with horseradish peroxidase-conjugated streptavidin (1:2000-dilution). After 30 minutes, the membranes were washed thoroughly and exposed to a chemiluminescent peroxidase substrate for 5 min in the dark before imaging. Membranes were exposed to x-ray film (Research Products International, Mt Prospect, IL). As per the manufacturer's package insert, the cytokine array data on developed X-ray film was quantitated by scanning the film on a transmission-mode scanner and analyzing the array image file using image analysis software, Un-Scan-it gel digitizing software version 6.1 (Silk Scientific Corporation, Orem, UT). Positive controls at three spots were used to identify membrane orientation and to normalize the results from different membranes. For each spot, the specific pixel level was determined by subtracting the background pixels from the total raw pixel levels. To quantify relative change in cytokine levels between

samples, the average background-subtracted mean spot pixel densities of the pair of duplicate spots representing each cytokine was determined for each condition. To facilitate further analyses, all spots in the arrays were quantified and their specific intensity values were obtained by subtracting the background intensity. Only differences in cytokine levels that were ≥ 2-fold compared to controls were considered significant.

Following incubation of the B cell lines with tumor-derived exosomes, total RNA from the exosome-treated and untreated B cells were extracted by Trizol. The profiles of 88 specific RNAs, associated with lymphocyte activation and function were analyzed in each population using RT-PCR arrays (Qiagen).

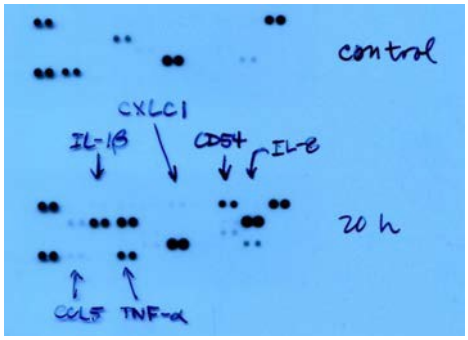


Figure: Representation cytokine array showing the production of specific cytokines and chemokines in untreated B cells compared to B cells exposed to 100ug tumor exosomes for 20 hours.

Based on the cytokine/chemokine arrays, exposure of B cells to tumor-derived exosomes induced those cytokines associated with B cell activation, but not T cell activation. Additional analyses of mRNA associated with exosome-treated B cells (Attachment 4) revealed that:

In RA-1 cells exposed to tumor exosomes for 5h and 16 h -
- exosome exposure increased expression of IL-10
- exosome exposure enhancedexpression of IL-6
- in the presence of CD40L and IL-4, exosomes exhibited an additional enhancement in IL-6 expression
- at 5h, the presence of tumor exosomes elevated expression of AID.

In H929 cells exposed to tumor exosomes for 5h and 16h -
- exosomes increased expression of IL-10, with an additional increase observed at 16h
- while the addition of CD40L alone increased expression of IL-10, the further addition of exosomes induced a greater enhancement of IL-10 expression
- exosomes at 5h elevated expression of IL-4

Under these conditions, genes associated with T cell activation were either suppressed or unchanged.

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ATTACHMENT 1

		M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12
Position	Well	Ct (dRn)	Ct (dRn)	Ct (dRn)	Ct (dRn)	Ct (dRn)	Ct (dRn)	Ct (dRn)	Ct (dRn)	Ct (dRn)	Ct (dRn)	Ct (dRn)	Ct (dRn)
A01	let-7a	17.78	14.44	13.02	12.93	12.46	12.74	14.88	14.36	12.86	11.58	13.81	12.91
A02	miR-133b	29.20	18.54	15.23	15.48	14.07	17.10	18.04	26.96	14.65	11.99	26.34	16.25
A03	miR-122	31.81	18.67	15.77	16.13	14.08	17.32	17.06	29.35	14.99	13.09	29.39	16.26
A04	miR-20b	20.90	15.99	13.67	15.05	14.06	15.00	16.74	17.90	14.18	12.03	17.22	14.18
A05	miR-335	26.91	18.52	14.70	15.89	14.55	17.04	17.24	23.61	15.11	12.88	22.87	15.68
A06	miR-196a	25.19	17.49	15.59	16.79	14.11	16.72	18.10	21.89	14.42	12.97	21.59	15.65
A07	miR-125a-5p	16.73	13.98	12.76	13.16	12.32	12.43	13.20	13.76	12.39	11.60	14.00	12.87
A08	miR-142-5p	32.45	16.88	14.55	16.04	13.41	16.39	16.66	29.30	14.19	12.16	29.40	14.27
A09	miR-96	21.19	15.12	13.31	13.72	12.82	13.92	14.89	17.38	13.48	11.36	17.04	13.65
A10	miR-222	27.30	16.62	14.75	14.17	12.60	15.77	15.45	23.24	13.88	11.17	20.73	14.49
A11	miR-148b	19.66	14.95	13.40	13.41	12.22	13.68	15.32	16.32	12.91	11.37	15.43	13.35
A12	miR-92a	19.28	14.41	12.96	13.22	12.33	13.64	14.50	15.79	12.74	11.17	14.99	13.12
B01	miR-184	30.84	30.32	28.42	28.41	28.34	27.81	29.82	27.59	28.24	27.74	27.72	28.03
B02	miR-214	31.43	30.29	29.36	29.97	29.89	29.99	32.89	29.19	30.65	29.35	28.65	30.17
B03	miR-15a	22.06	20.39	18.87	18.85	19.13	18.17	24.68	18.53	19.04	17.96	18.19	19.00
B04	miR-18b	27.94	26.45	25.27	24.27	25.74	25.38	27.68	23.82	30.47	24.40	23.13	25.40
B05	miR-378	20.27	18.19	17.19	17.39	16.94	16.11	19.73	16.46	17.10	16.97	16.62	16.88
B06	let-7b	20.23	18.96	17.28	16.67	17.17	16.40	20.91	16.75	16.93	16.55	16.12	16.89
B07	miR-205	23.15	22.06	20.82	20.81	21.74	19.77	24.13	19.94	20.06	20.03	20.13	20.49
B08	miR-181a	20.05	18.80	17.35	16.93	18.11	16.81	22.00	16.84	16.54	16.25	16.92	17.10
B09	miR-130a	26.32	25.63	24.10	23.45	24.77	23.16	29.50	23.06	23.59	22.97	23.28	23.38
B10	miR-199a-3p	26.54	24.52	23.21	23.83	22.93	22.24	25.69	23.16	23.21	23.08	23.47	23.54
B11	miR-140-5p	24.06	22.52	20.45	20.87	20.91	19.50	25.30	20.14	20.86	20.05	20.08	20.95
B12	miR-20a	19.11	17.66	15.88	15.30	15.61	14.92	20.23	15.52	17.68	15.05	15.26	15.63
C01	miR-146b-5p	22.90	21.08	19.66	19.84	19.41	18.75	21.22	19.62	19.55	19.07	19.40	19.96
C02	miR-132	25.63	24.22	22.13	20.95	21.07	20.93	24.95	22.02	21.32	20.80	21.08	21.11
C03	miR-193b	19.71	17.86	16.93	16.70	17.23	16.43	20.73	17.09	16.55	16.40	16.56	16.86
C04	miR-183	20.36	18.07	17.05	16.85	17.00	16.07	18.27	17.04	16.56	16.36	16.62	16.73
C05	miR-34c-5p	30.18	28.90	27.74	26.82	27.83	27.49	30.13	26.84	28.86	26.77	26.30	27.71
C06	miR-30c	19.54	17.51	16.86	16.73	17.19	16.06	19.20	16.19	16.51	15.97	16.07	16.37
C07	miR-148a	19.38	17.35	16.28	15.40	15.73	15.25	31.03	15.84	15.92	15.14	15.08	15.82
C08	miR-134	32.91	30.59	29.68	30.27	30.33	29.46	30.74	29.06	30.05	29.62	26.85	30.23
C09	let-7g	19.57	17.70	16.09	15.98	16.01	15.41	19.80	15.87	16.19	15.33	15.42	16.25
C10	miR-138	30.23	28.84	27.99	27.39	28.08	27.09	31.01	27.01	27.69	26.01	25.68	27.88
C11	miR-373	31.84	31.19	29.29	29.36	30.36	30.84	32.11	28.70	30.25	29.97	28.11	31.90
C12	let-7c	23.30	22.10	21.56	20.58	21.10	19.71	23.65	20.05	21.09	19.93	19.66	20.40
D01	let-7e	18.34	16.71	15.47	15.23	15.16	14.39	17.98	14.74	15.44	14.38	14.31	15.05
D02	miR-218	24.33	22.40	21.25	21.22	20.94	19.61	25.34	20.40	20.85	20.47	21.10	21.59
D03	miR-29b	26.95	24.18	23.56	24.49	24.56	23.34	29.31	23.27	25.57	22.72	23.55	24.87
D04	miR-146a	31.95	30.68	29.17	28.71	29.55	28.27	30.42	29.02	29.81	28.40	28.52	29.27
D05	miR-212	28.19	26.04	24.99	25.45	26.25	25.98	27.72	24.80	28.27	25.78	25.06	26.15
D06	miR-135b	26.73	24.51	22.83	21.31	21.80	22.71	27.18	22.95	23.43	22.18	21.43	22.95
D07	miR-206	31.40	30.12	28.95	29.59	29.52	28.96	31.50	27.97	29.88	29.46	27.73	29.88
D08	miR-124	28.84	26.58	26.01	26.35	28.33	26.45	29.01	26.10	26.94	25.65	25.58	26.76
D09	miR-21	13.29	11.62	10.68	10.64	9.68	10.72	11.77	9.98	10.60	9.84	9.83	10.80
D10	miR-181d	22.80	21.55	20.04	20.06	20.26	19.25	24.09	19.32	19.93	19.06	19.49	19.87
D11	miR-301a	19.92	18.19	16.82	16.41	16.74	15.76	23.35	16.31	16.90	15.62	15.38	16.88
D12	miR-200c	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct
E01	miR-100	25.51	24.48	23.52	23.28	23.40	22.08	24.25	22.74	23.69	22.76	23.13	23.46
E02	miR-10b	29.47	28.13	27.07	27.32	27.08	26.33	29.04	26.77	28.10	27.10	26.62	27.20
E03	miR-155	31.20	29.27	28.79	28.15	28.17	27.80	30.44	28.09	28.75	28.62	27.44	28.47
E04	miR-1	31.19	29.27	28.18	28.29	27.67	26.83	30.91	27.81	28.96	28.17	27.86	29.41
E05	miR-363	28.36	26.08	25.34	24.75	25.22	24.14	29.09	25.33	24.79	24.54	24.77	25.05
E06	miR-150	30.04	28.33	27.50	27.33	27.16	25.88	28.58	26.53	25.91	26.99	26.75	26.48
E07	let-7i	19.42	17.27	16.35	15.63	16.04	15.60	20.26	15.82	16.07	15.08	15.22	15.59
E08	miR-27b	18.01	15.64	14.39	14.75	14.62	14.08	16.67	14.56	14.43	14.00	14.12	14.87
E09	miR-7	18.96	16.82	15.48	15.79	15.32	14.67	17.44	15.31	16.13	15.23	15.11	16.60
E10	miR-127-5p	28.12	26.14	23.42	25.11	25.95	26.05	25.25	24.71	28.82	24.56	20.34	27.13
E11	miR-29a	20.73	19.07	17.85	17.09	17.19	16.53	22.86	16.57	17.69	17.15	16.76	17.01
E12	miR-191	16.68	14.57	14.11	13.58	13.47	12.83	14.99	13.50	13.50	13.29	13.36	13.21
F01	let-7d	20.41	18.64	17.39	16.89	17.72	16.83	21.10	17.84	17.57	16.74	16.57	16.89
F02	miR-9	23.87	21.63	20.31	20.23	19.98	19.24	21.59	20.25	21.01	19.91	20.25	20.80
F03	let-7f	24.54	22.81	21.65	22.11	20.77	20.77	23.80	20.96	24.06	21.05	20.70	22.20
F04	miR-10a	28.50	27.34	26.30	26.30	26.20	25.13	25.59	26.04	26.50	26.82	25.76	26.68
F05	miR-181b	20.02	18.24	17.09	17.24	17.47	16.35	20.18	16.61	16.75	16.57	16.82	17.01
F06	miR-15b	17.49	15.29	14.51	13.88	14.29	13.57	15.40	14.07	14.05	13.28	13.87	13.92
F07	miR-16	15.52	13.77	12.60	12.39	11.87	11.93	14.48	12.03	12.34	12.12	11.95	12.47
F08	miR-210	25.24	22.73	21.60	21.53	22.19	21.85	26.30	21.33	22.13	20.61	19.78	20.95
F09	miR-17	19.33	17.69	16.19	15.38	15.95	15.40	20.55	15.45	15.78	15.48	15.10	15.63
F10	miR-98	22.08	19.95	18.65	18.66	18.02	17.37	21.34	17.83	19.19	17.80	18.12	18.99
F11	miR-34a	23.52	22.00	20.82	19.53	20.83	19.01	25.69	19.06	20.60	18.61	18.61	19.59

F12	miR-25	17.65	15.77	14.69	14.06	14.20	13.76	15.29	14.22	14.51	13.60	14.29	14.04
G01	miR-144	35.77	34.55	32.97	32.19	No Ct	32.65	37.18	31.00	34.64	31.85	30.80	33.58
G02	miR-128	21.37	19.17	18.51	18.29	17.87	17.23	19.57	17.34	18.19	17.81	17.76	17.97
G03	miR-143	29.25	27.53	26.10	25.80	28.15	25.82	29.36	25.09	27.35	25.33	25.02	26.72
G04	miR-215	29.84	27.93	26.60	26.62	25.44	25.34	28.04	25.73	26.58	26.26	26.04	26.42
G05	miR-19a	19.52	17.32	15.88	15.35	15.81	15.32	22.24	15.61	16.00	15.08	15.21	16.12
G06	miR-193a-5p	26.35	24.89	23.74	23.48	24.07	22.73	26.53	23.34	23.11	23.42	22.06	23.73
G07	miR-18a	21.91	20.29	18.58	18.10	18.16	17.54	23.72	17.45	18.73	16.79	17.54	18.11
G08	miR-125b	20.73	19.06	19.74	18.38	18.41	17.05	17.93	17.46	18.03	17.45	18.05	18.12
G09	miR-126	20.93	19.09	17.87	18.02	17.86	16.82	20.70	17.55	17.72	17.29	17.71	17.76
G10	miR-27a	17.89	15.84	14.66	14.24	14.01	13.72	16.60	14.06	14.18	13.72	13.56	14.21
G11	miR-372	28.08	26.89	25.27	26.42	27.05	26.86	33.69	25.35	26.89	25.94	23.47	27.46
G12	miR-149	25.77	22.84	22.69	23.43	23.42	21.93	23.57	21.71	22.63	22.84	22.98	23.05
H01	miR-23b	19.15	16.58	15.56	15.63	15.81	15.42	17.36	15.33	15.50	15.20	15.33	15.79
H02	miR-203	19.91	18.64	16.90	16.56	16.28	15.75	17.26	16.67	16.82	16.34	16.54	16.71
H03	miR-32	28.17	24.55	24.78	25.21	25.53	24.34	29.12	24.18	26.17	24.44	24.66	25.90
H04	miR-181c	20.83	19.47	18.23	18.02	18.74	17.52	22.78	17.15	17.74	17.60	17.37	17.87
H05	SNORD48	26.38	24.49	23.68	23.27	23.66	22.77	27.38	22.50	24.37	22.44	21.47	22.72
H06	SNORD47	19.74	16.95	16.98	16.35	16.41	15.78	18.35	14.44	16.36	15.85	15.42	16.52
H07	SNORD44	15.99	13.64	13.15	12.88	12.55	12.46	14.03	12.37	12.78	12.40	12.17	12.50
H08	U6	21.98	18.38	19.21	19.86	19.11	19.00	23.49	17.25	19.38	18.81	16.90	17.79
H09	miRTC	22.22	18.82	18.54	19.06	19.14	18.64	23.55	19.05	19.18	19.02	18.73	18.86
H10	miRTC	22.28	18.73	18.56	18.88	19.12	18.60	23.29	18.86	19.14	18.61	19.46	19.12
H11	PPC	19.49	16.16	15.80	15.82	16.14	15.70	15.54	16.13	15.79	16.17	15.49	15.81
H12	PPC	19.40	16.23	15.75	16.08	16.20	15.77	15.79	16.18	15.93	16.34	15.75	15.80

ATTACHMENT 2

PCR Array Catalog #:		MAH-102					
				Control	Group 1	Group 2	Group 3
Position	**Mature ID**	**Accession Number**	**miRNA Catalog**	**HUMAN AB-2**	**1211463 (bladder)**	**1449338 (colon)**	**1622634 (pancreas)**
A01	let-7a	MIMAT0000062	MPH00001A	Ct (dRn)	Ct (dRn)	Ct (dRn)	Ct (dRn)
A02	miR-133b	MIMAT0000770	MPH00033A	35.50	Undetermined	22.44	19.35
A03	miR-122	MIMAT0000421	MPH00020A	33.86	Undetermined	21.4	20.14
A04	miR-20b	MIMAT0001413	MPH00105A	34.45	Undetermined	22.4	18.2
A05	miR-335	MIMAT0000765	MPH00166A	33.12	35.89	21.56	18.35
A06	miR-196a	MIMAT0000226	MPH00085A	38.25	Undetermined	22.8	20.38
A07	miR-125a-5p	MIMAT0000443	MPH00022A	34.93	38.38	21.83	19.74
A08	miR-142-5p	MIMAT0000433	MPH00043A	29.79	34.55	21.36	18.73
A09	miR-96	MIMAT0000095	MPH00479A	35.53	Undetermined	19.7	18.39
A10	miR-222	MIMAT0000279	MPH01230A	34.37	35.47	23.46	16.83
A11	miR-148b	MIMAT0000759	MPH01183A	34.15	36.84	20.9	17.53
A12	miR-92a	MIMAT0000092	MPH01375A	36.61	37.05	20.98	17.46
B01	miR-184	MIMAT0000454	MPH00070A	29.19	31.78	19.57	18.15
B02	miR-214	MIMAT0000271	MPH01223A	36.39	Undetermined	37.88	38.13
B03	miR-15a	MIMAT0000068	MPH00060A	31.35	34.58	35.41	34.88
B04	miR-18b	MIMAT0001412	MPH00076A	33.67	39.32	37.17	36.53
B05	miR-378	MIMAT0000732	MPH01284A	32.10	38.52	34.75	34.8
B06	let-7b	MIMAT0000063	MPH00002A	33.09	38.14	36.19	37.11
B07	miR-205	MIMAT0000266	MPH00100A	32.48	37.47	37.55	Undetermined
B08	miR-181a	MIMAT0000256	MPH00064A	32.53	39.54	36.41	36.36
B09	miR-130a	MIMAT0000425	MPH01165A	34.48	38.67	36.53	37.66
B10	miR-199a-3p	MIMAT0000232	MPH01212A	32.33	37.04	35.78	36.41
B11	miR-140-5p	MIMAT0000431	MPH00041A	35.71	Undetermined	Undetermined	39.31
B12	miR-20a	MIMAT0000075	MPH00104A	37.99	Undetermined	38.09	Undetermined
C01	miR-146b-5p	MIMAT0002809	MPH00048A	33.48	35.87	36.23	37.09
C02	miR-132	MIMAT0000426	MPH01167A	35.31	Undetermined	36.56	37.2
C03	miR-193b	MIMAT0002819	MPH01208A	31.86	36.01	35.56	34.51
C04	miR-183	MIMAT0000261	MPH00069A	31.39	35.85	34.93	35.67
C05	miR-34c-5p	MIMAT0000686	MPH00178A	33.49	39.66	Undetermined	Undetermined
C06	miR-30c	MIMAT0000244	MPH00152A	30.08	34.91	35.44	35.29
C07	miR-148a	MIMAT0000243	MPH01182A	34.79	Undetermined	Undetermined	Undetermined
C08	miR-134	MIMAT0000447	MPH00034A	34.34	36.75	37.15	38.68
C09	let-7g	MIMAT0000414	MPH00007A	36.06	38.68	39.47	Undetermined
C10	miR-138	MIMAT0000430	MPH00039A	Undetermined	Undetermined	Undetermined	39.21
C11	miR-373	MIMAT0000726	MPH01280A	32.74	38.17	36.34	36.2
C12	let-7c	MIMAT0000064	MPH00003A	33.26	38.96	37.83	37.57
D01	let-7e	MIMAT0000066	MPH00005A	35.89	Undetermined	39.19	Undetermined
D02	miR-218	MIMAT0000275	MPH00115A	34.52	37.93	39.35	Undetermined
D03	miR-29b	MIMAT0000100	MPH01245A	33.48	Undetermined	Undetermined	Undetermined
D04	miR-146a	MIMAT0000449	MPH00047A	35.77	Undetermined	Undetermined	39.56
D05	miR-212	MIMAT0000269	MPH00109A	35.38	Undetermined	37.57	38.73
D06	miR-135b	MIMAT0000758	MPH00036A	29.46	33.56	32.63	32.08
D07	miR-206	MIMAT0000462	MPH00101A	32.04	37.42	32.42	38.7
D08	miR-124	MIMAT0000422	MPH01157A	32.18	Undetermined	36.04	34.98
D09	miR-21	MIMAT0000076	MPH00106A	28.03	32.08	32.71	31.97
D10	miR-181d	MIMAT0002821	MPH00067A	31.46	34.45	38.19	38.94
D11	miR-301a	MIMAT0000688	MPH00144A	34.09	36.91	38.92	37.47
D12	miR-200c	MIMAT0000617	MPH01218A	33.61	35.88	37.45	36.19
E01	miR-100	MIMAT0000098	MPH00009A	33.95	35.95	34.03	36.92
E02	miR-10b	MIMAT0000254	MPH00018A	34.67	38.67	37.77	39.06
E03	miR-155	MIMAT0000646	MPH00059A	33.98	Undetermined	38.34	38.01
E04	miR-1	MIMAT0000416	MPH00019A	35.82	39.44	Undetermined	39.52
E05	miR-363	MIMAT0000707	MPH01276A	29.69	Undetermined	35.7	Undetermined
E06	miR-150	MIMAT0000451	MPH00054A	33.99	37.94	38.94	Undetermined
E07	let-7i	MIMAT0000415	MPH00008A	32.52	Undetermined	39.7	34.35
E08	miR-27b	MIMAT0000419	MPH01240A	33.31	36.95	33.42	36.57
E09	miR-7	MIMAT0000252	MPH00427A	34.55	Undetermined	Undetermined	Undetermined
E10	miR-127-5p	MIMAT0004604	MPH00025A	30.90	36.62	35.36	36.64
E11	miR-29a	MIMAT0000086	MPH01244A	32.81	Undetermined	38.49	38.89
E12	miR-191	MIMAT0000440	MPH00079A	32.70	38.08	37.57	36.85
F01	let-7d	MIMAT0000065	MPH00004A	31.55	38.35	35.18	36.28
F02	miR-9	MIMAT0000441	MPH00456A	33.51	35.41	36.42	36.45
F03	let-7f	MIMAT0000067	MPH00006A	33.08	38.8	34.89	37.28
F04	miR-10a	MIMAT0000253	MPH00017A	36.92	Undetermined	Undetermined	Undetermined
F05	miR-181b	MIMAT0000257	MPH00065A	34.35	Undetermined	37.44	38.43
F06	miR-15b	MIMAT0000417	MPH00061A	28.34	33.86	32.8	33.34
F07	miR-16	MIMAT0000069	MPH00062A	33.22	34.55	Undetermined	39.48
F08	miR-210	MIMAT0000267	MPH00107A	31.59	34.27	35.81	36.38
F09	miR-17	MIMAT0000070	MPH00063A	27.07	31.96	30.31	31.65
F10	miR-98	MIMAT0000096	MPH00480A	31.49	34.95	33.35	34.63
F11	miR-34a	MIMAT0000255	MPH00176A	36.27	Undetermined	Undetermined	Undetermined
F12	miR-25	MIMAT0000081	MPH01235A	33.06	36.75	35.4	37.82
G01	miR-144	MIMAT0000436	MPH01178A	32.07	33.65	34.77	37.84
G02	miR-128	MIMAT0000424	MPH00026A	37.59	Undetermined	Undetermined	Undetermined
G03	miR-143	MIMAT0000435	MPH01177A	34.29	39.33	38.83	Undetermined
G04	miR-215	MIMAT0000272	MPH00111A	33.25	37.28	33.24	37.79
G05	miR-19a	MIMAT0000073	MPH01214A	35.15	Undetermined	38.14	Undetermined
G06	miR-193a-5p	MIMAT0004614	MPH00081A	32.36	38.39	33.91	36.64
G07	miR-18a	MIMAT0000072	MPH00075A	30.36	34.2	33.94	32.81
G08	miR-125b	MIMAT0000423	MPH00023A	32.58	36.22	36.56	36.16
G09	miR-126	MIMAT0000445	MPH01161A	30.75	35.39	31.16	34.64
G10	miR-27a	MIMAT0000084	MPH01239A	31.99	39.65	34.38	Undetermined
G11	miR-372	MIMAT0000724	MPH00187A	32.48	38.7	37.57	38.49

G12	miR-149	MIMAT0000450	MPH00053A	29.42	33.63	34.33	31.51
H01	miR-23b	MIMAT0000418	MPH01233A	33.04	36.89	35.92	36.02
H02	miR-203	MIMAT0000264	MPH00098A	32.64	36.3	37.47	Undetermined
H03	miR-32	MIMAT0000090	MPH00156A	32.34	35.51	34.37	38.78
H04	miR-181c	MIMAT0000258	MPH00066A	36.24	Undetermined	Undetermined	Undetermined
H05	SNORD48	NR_002745	MPH01657A	30.43	31.65	30.36	31.16
H06	SNORD47	NR_002746	MPH01660A	28.60	33.58	32.91	34.74
H07	SNORD44	NR_002750	MPH01658A	34.69	39.9	39.16	Undetermined
H08	RNU6-2	NR_002752	MPH01653A	35.10	Undetermined	Undetermined	Undetermined
H09	miRTC	SA_miRNA_005	MPH01656A	30.32	37.22	34.52	32.97
H10	miRTC	SA_miRNA_005	MPH01656A	18.47	23.35	18.57	21.44
H11	PPC	SA_00104	PPX63339A	18.41	23.04	18.62	20.97
H12	PPC	SA_00104	PPX63339A	16.64	17.28	17.92	17.23
				16.67	17.44	17.94	16.89

ATTACHMENT 3a

	Symbol	Description	Control	Exo-Treated
A01	ADA	Adenosine deaminase	22.26	18.59
A02	AICDA	Activation-induced cytidine deaminase	35	19.52
A03	APC	Adenomatous polyposis coli	29.04	17.34
A04	BCL2	B-cell CLL/lymphoma 2	26.64	18.84
A05	BLM	Bloom syndrome, RecQ helicase-like	25.9	20.27
A06	BLNK	B-cell linker	29.62	18.43
A07	CCL3	Chemokine (C-C motif) ligand 3	20.25	18.2
A08	CCR1	Chemokine (C-C motif) receptor 1	22.74	17.38
A09	CCR2	Chemokine (C-C motif) receptor 2	34.57	20.14
A10	CCR3	Chemokine (C-C motif) receptor 3	30.74	19.21
A11	CCR4	Chemokine (C-C motif) receptor 4	33.39	22.08
A12	CCR5	Chemokine (C-C motif) receptor 5	32.92	17.54
B01	CD1D	CD1d molecule	27.58	35
B02	CD2	CD2 molecule	30.68	35
B03	CD27	CD27 molecule	32.64	35
B04	CD274	CD274 molecule	30.97	35
B05	CD276	CD276 molecule	26.49	35
B06	CD28	CD28 molecule	22.36	35
B07	CD3D	CD3d molecule, delta (CD3-TCR complex)	35	35
B08	CD3E	CD3e molecule, epsilon (CD3-TCR complex)	25.73	35
B09	CD3G	CD3g molecule, gamma (CD3-TCR complex)	33.33	35
B10	CD4	CD4 molecule	31.43	35
B11	CD40	CD40 molecule, TNF receptor superfamily member 5	29.23	35
B12	CD40LG	CD40 ligand	32.39	30.63
C01	CD47	CD47 molecule	22.98	35
C02	CD5	CD5 molecule	35	35
C03	CD7	CD7 molecule	30.83	34.36
C04	CD80	CD80 molecule	33.14	35
C05	CD81	CD81 molecule	23.04	35
C06	CD86	CD86 molecule	27.56	35
C07	CD8A	CD8a molecule	32.92	35
C08	CD8B	CD8b molecule	35	35
C09	CSF2	Colony stimulating factor 2 (granulocyte-macrophage)	29.81	32.71
C10	CX3CL1	Chemokine (C-X3-C motif) ligand 1	33.8	32.65
C11	CXCR3	Chemokine (C-X-C motif) receptor 3	27.67	34.49
C12	CXCR4	Chemokine (C-X-C motif) receptor 4	35	35
D01	CXCR5	Chemokine (C-X-C motif) receptor 5	32.24	35
D02	DPP4	Dipeptidyl-peptidase 4	32.49	35
D03	EGR1	Early growth response 1	29.28	35
D04	FAS	Fas (TNF receptor superfamily, member 6)	25.55	35
D05	FASLG	Fas ligand (TNF superfamily, member 6)	31.91	35
D06	FOXP3	Forkhead box P3	28.83	34.15
D07	ICOSLG	Inducible T-cell co-stimulator ligand	27.24	35
D08	IFNG	Interferon, gamma	32.47	35
D09	IL10	Interleukin 10	27.4	35
D10	IL11	Interleukin 11	33.88	35
D11	IL12A	Interleukin 12A (natural killer cell stimulatory factor 1, cyto	26.47	35

D12	IL12B	Interleukin 12B (natural killer cell stimulatory factor 2, cyto	35	35
E01	IL12RB1	Interleukin 12 receptor, beta 1	25.13	33.29
E02	IL12RB2	Interleukin 12 receptor, beta 2	28.17	35
E03	IL13	Interleukin 13	30.04	33.41
E04	IL15	Interleukin 15	27.78	35
E05	IL18	Interleukin 18 (interferon-gamma-inducing factor)	30.9	34.26
E06	IL18R1	Interleukin 18 receptor 1	32.58	35
E07	IL1B	Interleukin 1, beta	33.33	35
E08	IL2	Interleukin 2	35	34.79
E09	IL2RA	Interleukin 2 receptor, alpha	35	35
E10	IL3	Interleukin 3 (colony-stimulating factor, multiple)	32.12	35
E11	IL4	Interleukin 4	33.1	35
E12	IL4R	Interleukin 4 receptor	27.09	35
F01	IL5	Interleukin 5 (colony-stimulating factor, eosinophil)	32.61	35
F02	IL6	Interleukin 6 (interferon, beta 2)	34.2	35
F03	IL7	Interleukin 7	35	35
F04	IL8	Interleukin 8	29.15	35
F05	IRF4	Interferon regulatory factor 4	20.03	35
F06	LAG3	Lymphocyte-activation gene 3	35	35
F07	LCK	Lymphocyte-specific protein tyrosine kinase	31.16	35
F08	MAP3K7	Mitogen-activated protein kinase kinase kinase 7	24.42	35
F09	MICB	MHC class I polypeptide-related sequence B	24.1	33.52
F10	MS4A1	Membrane-spanning 4-domains, subfamily A, member 1	35	35
F11	NCK1	NCK adaptor protein 1	24.92	35
F12	NOS2	Nitric oxide synthase 2, inducible	33.92	35
G01	PTPRC	Protein tyrosine phosphatase, receptor type, C	30.72	35
G02	RAG1	Recombination activating gene 1	34.2	35
G03	RIPK2	Receptor-interacting serine-threonine kinase 2	26.42	35
G04	SOCS1	Suppressor of cytokine signaling 1	27.94	35
G05	TGFB1	Transforming growth factor, beta 1	20.78	35
G06	TLR1	Toll-like receptor 1	32.47	35
G07	TLR2	Toll-like receptor 2	35	35
G08	TLR4	Toll-like receptor 4	26.03	35
G09	TLR6	Toll-like receptor 6	29.3	35
G10	TLR9	Toll-like receptor 9	33.12	35
G11	TNFSF14	Tumor necrosis factor (ligand) superfamily, member 14	34.71	34.51
G12	VAV1	Vav 1 guanine nucleotide exchange factor	22.33	35
H01	ACTB	Actin, beta	18.69	35
H02	B2M	Beta-2-microglobulin	19.19	32.5
H03	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	19.81	32.64
H04	HPRT1	Hypoxanthine phosphoribosyltransferase 1	23.65	35
H05	RPLP0	Ribosomal protein, large, P0	16.82	29.13
H06	HGDC	Human Genomic DNA Contamination	35	35
H07	RTC	Reverse Transcription Control	20.97	20.15
H08	RTC	Reverse Transcription Control	20.91	19.33
H09	RTC	Reverse Transcription Control	20.97	19.92
H10	PPC	Positive PCR Control	16.88	16.81
H11	PPC	Positive PCR Control	16.81	16.61

H12	PPC	Positive PCR Control	16.89	16.72

RELATIVE EXPRESSION NORMALIZED TO THE REFERENCE GENE (GAPDH)

	A	B	C	D	E	F
1	**SAMPLE**	**IL-4 rel exp**	**IL-6 rel exp**	**IL-10 rel exp**	**IgGH rel exp**	
2	C5h RA-1 alone	1.69	1.21	147	2500	
3	T5h (RA1+ exo)	1.22	2.73	1652		
4	T5h (+ IL-4)	1	1.92	427.6		
5	T5h (+ CD40L)	1.52	1.79	263.2		
6	T5h (ILCD)**off	1000	2000	27.8		
7	T5h (ALL)	2.71	9	1795.3	8.33	
8	C16h RA1 alone	1.82	1.93	1016.9		
9	T16h (RA1 + exo)	2.08	1.66	1509.7		
10	T16h (+ IL-4)	2	1.28	464.6		
11	T16h (+ CD40L)	2.37	2.85	1287		
12	T16h (ILCD)	3.16	1.46	96.3	16.9	
13	T16h (ALL)	1.65	3.58	2005	12.3	
14						
15	C5h H929 alone	6653	2402	4.69	102126	
16	T5h (H929 + exo)	596	243.9	4.32	4451.3	
17	T5h (+ IL-4)	3956	1596	2.68	97289.7	
18	T5h (+ CD40L)	855	421.6	1.34	11505.2	
19	T5h (ILCD)	8902.5	5000	65.8		
20	T5h (ALL)	43.1	54.2	13.5	4124	
21	C16h H929 alone	3350	1782.9	1.93	106463	
22	T16h (H929 + exo)	1.14	1.47	600	40.5	
23	T16h (+ IL-4)	13124.7	2665.1	7.57	207104.5	
24	T16h (+ CD40L)	1000	393	2.09	12077.2	
25	T16h (ILCD)	5792.6	10226.3	93.7		
26	T16h (ALL)	2.64	1.4	533.7	148.1	

ATTACHMENT 3b RA-1

RELATIVE EXPRESSION NORMALIZED TO THE REFERENCE GENE (GAPDH)

	G	H	I	J
1	**SAMPLE**	**RAG1 rel exp**	**RAG2 rel exp**	**AID rel exp**
2	C5h RA-1 alone	25.6	6.25	3.13
3	T5h (RA1+ exo)			2.28
4	T5h (+ IL-4)			2
5	T5h (+ CD40L)			1.85
6	T5h (ILCD)**off	1000	3333	
7	T5h (ALL)	1.13	12.2	9.92
8	C16h RA1 alone			3.44
9	T16h (RA1 + exo)			4.34
10	T16h (+ IL-4)			4.17
11	T16h (+ CD40L)			3.22
12	T16h (ILCD)	1.43		
13	T16h (ALL)	2.77	9.09	2.55
14				
15	C5h H929 alone	6653.9	6653.6	102126
16	T5h (H929 + exo)	3061.5	1261.2	1000
17	T5h (+ IL-4)	3125.8	4576.4	97289.7
18	T5h (+ CD40L)	560.3	1000	8902.5
19	T5h (ILCD)			
20	T5h (ALL)	809	3565.8	1488.9
21	C16h H929 alone	2352.5	3565.8	66913.1
22	T16h (H929 + exo)	13.3	16.6	27.5
23	T16h (+ IL-4)	5673.4	8719.3	829521.6
24	T16h (+ CD40L)	292	364.6	178.5
25	T16h (ILCD)			
26	T16h (ALL)	19.97	216.7	1.62